Mechanosensitive cardiac C-fiber response to changes in left ventricular filling, coronary perfusion pressure, hemorrhage, and volume expansion in rats

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Ditting, Tilmann, Karl F. Hilgers, Karie E. Scrogin, Alexander Stetter, Peter Linz, and Roland Veelken. Mechanosensitive cardiac C-fiber response to changes in left ventricular filling, coronary perfusion pressure, hemorrhage, and volume expansion in rats. Am J Physiol Heart Circ Physiol 288: H541–H552, 2005. First published October 7, 2004; doi:10.1152/ajpheart.00131.2004.—Left ventricular (LV) end-diastolic pressure (LVEDP) increase due to volume expansion (VExp) increases mechanosensitive vagal cardiac afferent C-fiber activity (CNFA), thus decreasing renal sympathetic nerve activity (RSNA). Hypotensive hemorrhage (hHem) attenuates RSNA despite decreased LVEDP. We hypothesized that CNFA increases with any change in LVEDP. Coronary perfusion pressure (CPP), supposedly affected in both conditions, might also be a stimulus of CNFA. VExp and hHem were performed in anesthetized male Sprague-Dawley rats while blood pressure, heart rate, and RSNA were measured. Cervical vagotomy abolished RSNA response in both reflex responses. Single-unit CNFA was recorded while LVEDP was changed. Rapid changes (±4, ±6, ±8 mmHg) were obtained by graded occlusion of the caval vein and descending aorta. Prolonged changes were obtained by VExp and hHem. Furthermore, CNFA was recorded in a modified Langendorff heart while CPP was changed (70, 100, 40 mmHg). Rapid LVEDP changes increased CNFA [caval vein occlusion: +16 ± 3 Hz (approximately +602%); aortic occlusion: +15 ± 3 Hz (approximately +553%); 70 units; P < 0.05]. VExp and hHem (n = 6) increased CNFA [VExp: +10 ± 4 Hz (approximately +1,033%); hHem: +10 ± 2 Hz (approximately +1,225%); P < 0.05]. An increase in CPP increased CNFA [+2 ± 1 Hz (approximately +225%); P < 0.05], whereas a decrease in CPP decreased CNFA [−0.8 ± 0.4 Hz (approximately −50%); P < 0.05]. All C fibers recorded originated from the left ventricle (LV). CNFA increased with any LVEDP change but changed equidirectionally with CPP. Thus neither LVEDP nor CPP fully accounts directly for afferent C-fiber and reflex sympathetic responses. The intrinsic afferent stimuli and receptive fields accounting for reflex sympathoinhibition still remain cryptic.

cardiac afferent C fiber; renal sympathetic nerve activity; cardiorenal reflexes

The sympathetic nervous system influences circulation and cardiac performance. Moreover, it controls volume homeostasis selectively via efferent renal sympathetic nerve activity (RSNA) (8, 43). Central sympathetic output is controlled by reflex mechanisms originating in the heart and lungs and in the blood vessels, referred to as cardiopulmonary and arterial baroreceptor reflex, respectively.

In the context of volume handling, cardiac C-fiber afferents traveling via the vagus nerve are a major determinant of central sympathetic output (9, 52). These fibers are thought to act mainly as tension receptors within the cardiac wall (38), and they are stimulated mechanically when cardiac filling pressure [left ventricular (LV) end-diastolic pressure (LVEDP)] increases in the setting of volume expansion. Within the nodose ganglion—where the cell bodies of these first-order sensory axons reside—mechanosensory information is relayed to the second neuron, traveling to the brain stem to be integrated. As a consequence, sympathetic nervous output is decreased to restore the baseline volume state (44, 47).

In rats, RSNA suppression due to volume load can be abolished by bilateral cervical vagotomy (Vx) (23, 44). In rabbits, it was shown that selective blockade of cardiac afferents by intrapericardially administered procaine abolished volume-induced renal sympathoinhibition as seen with Vx or when Vx was combined with sinoaortic denervation (3). These findings indicate that RSNA suppression due to volume expansion is predominantly mediated by cardiac vagal afferents. Sympathetic afferents seem not to be involved in this reflex response (16). They are thought to play a major role in cardiac pain (4).

On the other hand, acute and graded aortic obstruction used to increase LVEDP by some investigators increased cardiac vagal afferent nerve activity in single-unit recording experiments (31, 47). Thus volume expansion or alternative measures able to increase LVEDP, i.e., to simulate central volume loading, increase cardiac vagal mechanosensory activity.

In volume-retaining disorders such as heart failure, liver cirrhosis, and nephrotic syndrome and in some forms of hypertension, alterations of these reflex pathways may contribute to etiologically relevant sympathetic dysregulation (10, 22, 33, 45). However, the culprit disturbances are not clearly localized (i.e., afferent, central, efferent) in these studies. Under physiological conditions such as pregnancy, cardiac vagal afferents (originating from the atria) may participate in volume retention (24), but this is certainly not the only mechanism responsible for gestational volume expansion in rats. It must be mentioned, however, that both ventricular and atrial fibers might contribute to reflex regulation of RSNA and that the individual contribution cannot be clearly distinguished in some studies.

With severe volume depletion, i.e., hypotensive hemorrhage, the situation is less clear. In unanesthetized mammals, the reflex response to acute hypotensive hemorrhage shows a...
biphasic pattern. An initial phase of baroreflex-mediated sympathoexcitaiton is followed by a “second phase” comprising an abrupt drop of sympathetic vasoconstrictor drive and hemodynamic decompensation that might be triggered, at least in part, by a signal from cardiac vagal afferents (17, 18). The efferent limb of this reflex response has been extensively investigated. Central opioid and serotonin pathways in different brain stem regions seem to be involved in the second-phase sympathoinhibitory response (7, 21, 32, 35, 51) as well as peripheral and spinal cord opioid receptors (2, 5), because appropriate administration of receptor blockers blunted the response. Furthermore, RSNA response seems to be modulated by high vasopressin levels in the setting of hemorrhage, whereas vasopressin receptors exert an inhibitory action on RSNA and contribute to blood pressure recovery via V1 receptors but exerts an opposite stimulatory action on RSNA via V2 receptors (19).

Much less is known about the putative role of the first-order, i.e., sensing, neuron and the initiating stimulus for its activation in the setting of hemorrhage. Hemorrhage-induced sympathoinhibition can be abolished by Vx. On the basis of indirect evidence, “paradoxical” activation of mechanosensitive vagal cardiac afferents has been suggested in rats (36). This mechanical activation is deemed to be a paradox because fibers that normally increase firing rate in response to increasing LVEDP are likewise activated when cardiac filling is critically lowered. Electrophysiological evidence for this hypothesis comes from an earlier feline study from Oberg and Thoren (31, 38). They suggested that “vigorous contraction of the heart around an empty chamber” might constitute the intrinsic stimulus for such paradoxical ventricular mechanoreceptor behavior. They found that some ventricular vagal afferents showed a sudden increase in firing rate when cardiac filling became critically low due to graded caval vein obstruction to simulate central hypovolemia (31, 38). However, a more recent study in dogs using a sophisticated in vivo heart perfusion system cast some doubt on the “classic hypothesis” that high inotropic state in combination with low ventricular filling constitutes a relevant stimulus for ventricular mechanoreceptors (15).

Our study was done to systematically investigate the responses of cardiac vagal afferents due to increases and decreases in LVEDP and to test the hypothesis that cardiac C-fiber activity in rats is augmented both with increases and decreases of LVEDP. Thus hemorrhage-induced sympathoinhibition might originate from the responsiveness of the first-order neuron as seen with volume expansion.

To stimulate cardiac vagal afferents mechanically, we used measures able to increase or decrease LVEDP. Volume expansion and hypotensive hemorrhage served as “physiological stimuli,” to excite cardiac afferent vagal fibers and to confirm that RSNA suppression is mediated by vagal afferent traffic. To this end volume expansion- and hemorrhage-induced reflexes were studied in vagotomized and intact rats by measuring efferent RSNA, heart rate (HR), blood pressure, and LVEDP. Furthermore, we performed single-unit recordings of cardiac vagal afferent C fibers in the setting of hypotensive hemorrhage and volume expansion. Both stimuli had been widely used in previous studies (12, 19, 21, 28, 34, 35, 44). Because of the ensuing counterregulatory effects, however, volume expansion and hemorrhage were not done in the same animals. To investigate the effects of any LVEDP change in one and the same afferent fiber, we performed balloon obstruction of the caval vein and aorta to simulate the LVEDP effects of volume expansion and hemorrhage, i.e., central volume loading and depletio (31, 47). Because these maneuvers might affect coronary perfusion, we also did vagal C-fiber recordings in a modified Langendorff heart preparation in which coronary perfusion pressure (CPP) could be changed independently. Moreover, at least in dogs, there is some evidence that CPP might constitute a stimulus for cardiac afferent vagal nerve fibers (13, 49, 50).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) weighing 370–550 g were used. Rats were kept at 24 ± 2°C with a 12:12-h light-dark cycle. They were fed a standard rat diet (no. C-1000; Altromin, Lage, Germany) containing 0.2% sodium chloride by weight and were allowed free access to tap water. All procedures performed on animals were done in accordance with the guidelines of the American Physiological Society and were approved by the Experimental Animal Care Committee of the local government agency (Regierung von Mittelfranken, Ansbach, Germany).

General Procedures

Rats were anesthetized with thiobarbital sodium (Trapanal; Byk Gulden, Konstanz, Germany; 40 mg/kg ip). To avoid spontaneous movements, especially in rats prepared for cardiac C-fiber recordings, due to susceptibility of the recording setup to vibration, animals were paralyzed with succinylcholine hydrochloride (Sigma-Aldrich, Deisenhofen, Germany; 1 mg/min iv). To achieve uniform experimental conditions throughout the study, rats prepared for RSNA recordings were also paralyzed and ventilated with oxygen-enriched room air via an orotracheal cannula with a rodent ventilator (Hugo Sachs Elektronik, March-Hugstetten, Germany). Respirator settings were as follows: tidal volume 1.3 ml/100 g body wt, positive end-expiratory pressure 3 cmH2O, maximum pressure 12 cmH2O, rate 70–100 breaths/min. Stability of artificial ventilation was ensured intermittently by blood gas analysis (arterial blood gases: pH 7.41 ± 0.08, PO2 131 ± 18 mmHg, PCO2 39 ± 2 mmHg), and adjustments were made by changes in respiratory rate as needed. Ventilation pressures were kept constant to avoid interference with cardiac preload. Care was taken for stable PCO2 levels because hypercapnia is known to interfere with cardiac C-fiber activity (38). For surgical procedures as well as experimental protocols, animals were kept in deep- to medium-state surgical anesthesia (48). Before paralysis, adequacy of anesthesia was confirmed by the lack of cardiovascular response and movement due to painful stimuli (e.g., absence of pedal withdrawal reflex). During paralysis, the absence of cardiovascular responses due to painful stimuli (careful pricking of the nasal septum, surgical manipulation) was used to assess the plane of anesthesia. To this end, blood pressure and HR were monitored continuously. Appropriate testing was repeated every 15–30 min. When cardiovascular responses reemerged, supplemental doses of thiobarbital sodium (20 mg/kg ip) were administered. This was usually necessary when the experiments lasted >6 h.

Catheter Insertion and Vagotomy Procedures

In animals used for RSNA recording (protocols 1 and 2), a catheter placed in the right femoral artery was connected to a strain-gauge transducer (Stratham P23Db) to record arterial blood pressure and HR (pressure processor type 13-4615-52; Gould Instrument Systems, Valley View, OH). In a subset of rats (protocol 2), a left femoral artery catheter was inserted for blood withdrawal, i.e., hypotensive hemorrhage. Two femoral venous lines were inserted for continuous infusion of normal saline (2.2 ml·kg⁻¹·h⁻¹) to compensate for fluid
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loss due to surgery and perspiration or volume loading (protocol 1) and for administration of substances, respectively. In some rats (experimental groups in protocols 1 and 2), high cervical bilateral Vx was performed to interrupt vagal afferent nerve traffic. A ventral midline cervical cut was made to expose both vagus nerves. These were cut centrally just beneath the nodose ganglion while care was taken to conserve all other structures, with special regard to the aortic depressor nerves and all other nervous and vascular structures.

In animals used for cardiac C-fiber recording (protocols 3, 4, and 5), the catheter for blood pressure and HR measurement was inserted into the left carotid artery. Another line was inserted into the left external jugular vein for infusion of saline and administration of drugs. Via the right carotid artery, a micropipet catheter pressure transducer (model SPC320; Millar Instruments, Houston, TX) was advanced into the LV for the measurement of LVEDP.

In a subset of rats (protocol 3), a fluid-filled Silastic balloon catheter was advanced to the aortic arch via a right femoral approach. The catheter was used to modulate LV pressure by obstructing cardiac outflow (i.e., to increase LVEDP). Another balloon catheter inserted in the inferior caval vein was positioned well beneath the right atrium to modulate cardiac preload while not directly stimulating atrial fibers (38). Adequate positioning of balloons was verified postmortem.

In another subset of animals (protocols 4 and 5), an additional catheter was inserted into the right femoral artery or vein for withdrawal of blood and volume loading, respectively. In these rats, only the aortic balloon catheter was inserted to test for mechanosensitive response of the C fiber under investigation.

All rats used for cardiac C-fiber recordings underwent subdiaphragmatic Vx to abolish intestinal afferent vagal activity in order to facilitate identification of cardiac C fibers. Briefly, the esophagus was exposed through a left flank incision. All vagal fibers in the vicinity of the esophagus were cut, and the esophagus was cleaned from all adherent connective tissue. The flank incision was closed in layers. As above, this Vx procedure was done at least 2 h before the experiments were started.

RSNA Recording

RSNA recording was performed as described previously (12, 44). The left kidney was exposed through a flank incision, and a renal nerve bundle was dissected free from connecting tissue and placed on a bipolar stainless steel electrode (Cooner Wire, Chatsworth, CA). RSNA was amplified 10,000–50,000 times and filtered (low pass: −12 dB/octave, 1 kHz; high pass: −12 dB/octave, 100 Hz) with a band-pass amplifier (Bioelectric signal conditioner type 13-6615-58; Gould Instrument Systems). The signal was channeled to an analog-to-digital oscilloscope (HM 205-3; Hameg, Frankfurt, Germany) and an audio amplifier loudspeaker (AM8 audio monitor; Grass-Telefactor, West Warwick, RI) for visual and auditory examination. The quality of the RSNA signal was assessed by its pulse synchronous rhythmicity and by examining the magnitude of decrease elicited by ganglionic blockade with trimetaphan-camsylate (Arfonad, 10 mg/kg iv; Roche, Basel, Switzerland). When an optimal signal could be observed (noise-to-signal-ratio at least 1:4), the nerve bundle was fixed to the recording electrode with a silicone adhesive (Bisico S4i; Bielefelder DentalSilicone, Bielefeld, Germany). The electrode was tunneled to the neck, and the site was closed in layers. The RSNA signal was full-wave rectified and integrated over 1-s intervals with an integrator amplifier (type 13-4615-70; Gould Instrument Systems) and was additionally smoothed by low-pass filtering (−6 dB/octave; 0.1 Hz) with a direct current amplifier (7DA; Grass-Telefactor). Amplifier settings were documented for recalibration of measured baseline activity (in μV-s) to allow for comparison of baselines between groups and protocols. The integrated RSNA signal as well as mean arterial blood pressure (MAP) and HR were recorded on a polygraph (RS 3400; Gould) and additionally stored on a computer with an analog-to-digital converter board (DT 2861; Data Translation, Marlborough, MA) and appropriate software (PSW 6.0; Data Wave Technologies, Longmont, CO) for evaluation. Sampling rate was 200 Hz.

After surgery, animals were allowed to stabilize for at least 2 h before the experimental protocols were started.

Cardiac C-Fiber Recording

Vagal C-fiber recording was performed as previously described (46, 47). The right vagus nerve was centrally cut and dissected free in the neck for a length of 3–4 cm. The right clavicle was cut centrally to allow the placement of the bipolar stimulation electrode as far distant as possible from the bipolar stainless steel recording electrode connected to a high-impedance probe. The cranial portion of the nerve was placed on a small black glass dissection plate. The margin of the skin was raised, and liquid paraffin was poured on the area of dissection for electrical isolation and to avoid drying of the nerve. The nerve was divided longitudinally into several smaller strands that were dissected further on the glass plate with fine sharpened forceps (Dumont no. 5; NeoLab, Heidelberg, Germany) under binocular microscope (magnification ×30–60; Leitz-Wild, Munich, Germany) until single units could be functionally characterized when mounting the fibers to the recording electrode. Single-fiber activity was amplified (×10,000) and filtered (high pass, 100 Hz; low pass, 3,000 Hz; −12 dB/octave, respectively) with a computer-controlled band-pass amplifier system with a high-impedance probe (CyberAmp 320; Axon Instruments, Union City, CA).

C-fiber impulses (sampling rate 20,000 Hz) as well as blood pressure, HR, LVEDP, and ECG were digitized (200 Hz) and stored on a computer using a commercially available data analysis setup with an analog-to-digital converter and a software-implemented window discriminator (DataWave Technologies Software Services). After experiments were finished, stored fiber waveforms were fed into a “spike-sort” subroutine provided by the data analysis program. Single nerve units were distinguishable from each other by waveform parameters. A software-implemented ratemeter counted the spikes in second bins [nerve C-fiber activity; CNFA (spikes/s; Hz)].

Identification of Cardiac Mechanosensitive C Fibers

Conduction velocity was determined by measuring the latency of electrical stimulation of the vagus nerve via a bipolar stainless steel electrode at the caudal part of the nerve exposed in the neck with a Grass S88 stimulator (Grass Instruments) connected to a Grass SIU 5 stimulus isolation unit (parameters: stimulation duration 0.45 ms; maximum stimulus strength 60 V, rectangular). Stimulus output was triggered by programmed burst data acquisition (100-ms period) with a delay of 20 ms and a sampling rate of 20,000 Hz. The evoked potential was recorded, and the conduction distance between the stimulation cathode and recording electrode was measured. Only fibers with a conduction velocity of <2.5 m/s, characteristic of C fibers, were used (37, 41).

To ensure that the identified C fibers were of cardiac origin, we attempted to exclude vagal C fibers from other sources by the following measures. First, intestinal C fibers were excluded by subdiaphragmatic Vx. Second, fibers exhibiting a spontaneous breath synchronous activity (>70% of all fibers screened) were excluded. Third, to further exclude pulmonary origin, static hyperinflation of the lung was induced by occluding tracheal outlet on the rodent ventilator for two strokes. All fibers that did not respond to hyperinflation were then stimulated mechanically by aortic balloon obstruction to increase LVEDP by 6 mmHg. Fibers that showed an increase in activity were assumed to be mechanosensitive cardiac C fibers and underwent the experimental protocols described below. Fibers that were electrically silent under baseline conditions were also excluded. Thus the inclusion criteria were C-fiber conductance, cardiac but not intestinal or pulmonary origin, and increase in activity due to aortic obstruction. At the end of the C-fiber recording experiments, the thorax was opened...
and the receptive field of fibers under investigation was identified by mechanical probing of the heart with a small glass pipette.

**Modified Langendorff Heart Preparation**

The modified Langendorff preparation was adapted from a method previously described for rabbits to electrically stimulate cardiac multifiber nerves (30). Animals were anesthetized, and catheters were inserted in the carotid artery and jugular vein for the control of blood pressure and HR and for the administration of drugs during the surgical procedure. A Silastic balloon was inserted via the right femoral artery and advanced to the aortic arch. After insertion of the catheters, a midline cervical incision was made. The animals were paralyzed after the trachea was cut and intubated with a plastic tube, and ventilation was maintained with a rodent ventilator. The common carotid arteries were identified and isolated with sutures, and the right vagus nerve was dissected. A single cardiac vagal C-fiber unit was identified as in protocols 3–5. Instant aortic obstruction by inflation of the balloon was performed to make sure that the respective C fiber was mechanosensitive. To facilitate relocation of this fiber, surrounding strands were cut off, and the fiber was placed on a small black glass pad that was fixed to the cervical muscles and immersed with paraffin oil to avoid drying of the fiber. The midline cervical incision was extended down the front of the chest wall to expose the rib cage. The pectoral muscles were dissected on both sides to expose subclavian vessels that were tied off with sutures. The thoracic cavity was opened via incisions on both sides, and the anterior portion of the rib cage was removed, exposing the mediastinal contents. Ice-cold Krebs-Henseleit perfusion solution was applied to the surface of the beating heart as well as to the thoracic cavity to lower temperature and metabolic rate. Krebs-Henseleit perfusion solution was composed as follows (in mmol/L): 118.0 NaCl, 4.7 KCl, 1.64 MgSO4, 1.5 KH2PO4, 24.88 NaHCO3, 2.52 CaCl2, and 11.1 glucose. The rat was euthanized with an intravenous overdose of thiobarbital sodium together with 30 U of heparin. After the balloon catheter was withdrawn, the descending aorta was cannulated with a flanged 2-mm-diameter plastic tube through which ice-cold perfusion solution was injected. The perfusion catheter was fixed with sutures. Both carotid arteries were tied off. The pulmonary artery was cut, and both lungs were removed to allow flow of perfusate out of the heart. The vertebral column was dissected at the level of the 12th thoracic vertebra. The lower part of the body was removed. The preparation extending from the neck to the thorax was then quickly dissected from the rest of the surrounding tissues. In particular, the right vagus nerve was cleaned from surrounding tissue to avoid potassium block of the nerve fibers emerging from deteriorating tissue. Because it was not possible to maintain contact between the identified C fiber and the recording electrode during the final steps of the procedure, the fiber was placed on the glass pad, where it was protected against mechanical trauma (i.e., tension). The fibers could be visually relocated and recorded in a sufficient amount of cases. Paraffin oil was used for electrical isolation and to avoid drying. However, in some preparations fibers were lost. Some could not be relocated when they slipped off the pad, and others showed a poor signal-to-noise ratio when they were repositioned on the electrode. LV balloons to double-check for LV pressure sensitivity of fibers were not used. On one hand, we were not successful in combining nerve recordings with LV balloons in a sufficient number of cases; on the other hand, aortic obstruction and LV inflation are not necessarily interchangeable stimuli. Thus we assumed that visually relocated fibers would be those that were tested initially, and because of the inclusion criteria used they would be well comparable to those recorded in the other protocol. Retrograde perfusion was started at a constant perfusion pressure of 70 mmHg, which was adjusted by the gas pressure above the fluid level within the self-contained reservoir. Within the reservoir, the perfusion medium was gassed with carbogen (95% O2-5% CO2) with a sintered glass gas distributor that was connected to a system of tubing functioning on the principle of a Mariott’s bottle to allow exact adjustment of perfusion pressure. The pH of the medium was maintained at 7.4 by bubbling with carbogen and was continuously monitored by a microprocessor-controlled pH meter (WTW, Weilheim, Germany). The whole perfusion system was water jacketed and connected to a heating pump (Braun, Melsungen, Germany) to keep temperature at 37°C. Temperature was continuously controlled with a custom-made thermistor probe within the perfusion cannula close to the heart.

**Experimental Protocols**

**General procedures.** For each experimental protocol (1–6) described below, a separate group of rats was used. In vivo protocols commenced with baroreceptor loading and unloading procedures. Intravenous boluses of the α1-agonist methoxamine (10 μg) and the vasodilator sodium nitroprusside (1 μg) were given in randomized order with a recovery period of 15 min in between. Methoxamine bolus increased blood pressure, with consecutive decrease in HR and RSNA. Sodium nitroprusside induced the opposite effect. Under stable conditions (i.e., euvolemic and sufficient anesthesia)—and only then—the baroreceptor reflex responses of HR and RSNA were nearly similar in magnitude during either increases or decreases of MAP. Because euvolemia is the essential prerequisite for the investigation of circulatory reflex mechanisms, baroreceptor testing was done in advance of every in vivo protocol. Furthermore, baroreceptor loading and unloading procedures allowed verification of the integrity of arterial baroreceptor afferents in those rats that underwent bilateral cervical Vx. At the end of all experiments, rats were euthanized with an intravenous overdose of thiobarbital.

**RSNA Recording Protocols.** **PROTOCOL 1. RSNA: VOLUME EXPANSION ± VX.** Putative mechanosensitive cardiac afferents were stimulated by an increase in LVEDP accomplished by volume loading. Control rats (n = 6) were given normal saline intravenously (5% body wt within 15 min). This regimen has been shown to stimulate vagal afferent axons, thereby eliciting reflex decreases of RSNA without changing arterial pressure or HR (44). MAP, HR, and RSNA were recorded simultaneously.

In the experimental group (n = 6), interruption of vagal afferents was accomplished by bilateral Vx, whereas arterial baroreceptor afferents were kept intact. Baroreceptor loading and unloading (see General procedures) was performed to confirm the integrity of these afferents. The same volume expansion protocol was done. Bilateral Vx was expected to abolish volume-loading-induced suppression of RSNA while leaving the arterial baroreceptor response untouched. **PROTOCOL 2. RSNA: HEMORRHAGE ± VX.** Mechanosensitive cardiac afferents were stimulated by decreasing cardiac filling pressure due to hypotensive hemorrhage. To this end, blood was withdrawn from the arterial line with an infusion-withdrawal pump in controls (n = 6) to lower MAP by ~50 mmHg within 5–6 min (Harvard Apparatus, Edenbridge, Kent, UK). Respective lines and syringes had been heparinized previously to avoid clotting of the withdrawn blood. Withdrawal rate was adjusted by the observed decrease of MAP. Blood withdrawal was stopped after 5–6 min when the target blood pressure was obtained and anesthesia was maintained. Vx was then quickly performed over 15 min after hemorrhage was started. The experimental group (n = 6) underwent the same hypotensive protocol after bilateral Vx. Hemorrhage was expected to induce short-lived renal sympathoexcitation followed by sustained sympathoinhibition. Vx was expected to abolish the hemorrhage-induced sympathoinhibition while leaving the arterial baroreceptor response untouched as in protocol 1.

**Cardiac C-fiber recordings.** **PROTOCOL 3. CNFA: RAPID INCREASES AND DECREASES IN LVEDP.** The intention of this protocol was to investigate cardiac C-fiber responses due to very rapid changes in cardiac filling pressure (LVEDP) in both directions within one and the same afferent fiber. Forty rats were instrumented, and cardiac mechanosensitive C fibers were identified by the maneuvers described above. LVEDP and cardiac afferent activity (CNFA) were recorded.
When a cardiac mechanosensitive C fiber was identified, fluid-filled balloons positioned in the aorta or caval vein were inflated gradually over 7–12 s to obtain maximal LVEDP changes of ±4, ±6, and ±8 mmHg from baseline, respectively, held inflated for 12–15 s, and deflated thereafter over 7–12 s. This was done by filling the balloons with a manual microinjector (Heka Elektronik, Lambrecht, Germany). Required volumes were determined and standardized in preliminary experiments (data not shown). These “one-on-one ramps” to obtain maximal LVEDP changes to either +4, +6, and +8 mmHg or −4, −6, and −8 mmHg from baseline were done separately, in randomized order, with recovery intervals of at least 5 min in between and then repeated in reversed order. Thus each afferent fiber investigated (i.e., fibers that passed the inclusion criteria, see Identification of Cardiac Mechanosensitive C Fibers) underwent up to 12 separate “ramps” when stable conditions could be maintained long enough. This experimental design was adapted from that of a recent study from our laboratory (47) that allowed for accurate adjustment of target LVEDP with rather linear increases or decreases. “Peak” responses were obtained while balloons were kept inflated. Increases in LVEDP were within the range of changes in pathophysiological situations, e.g., heart failure; furthermore, they were well within the range of LVEDP changes that were induced by the volume loading protocol. Decreases were in part well below the “physiological range.” At the end of the experiment, rats were euthanized with an overdose of thiobarbital, the thorax was opened, and the receptive field of the cardiac C fibers under investigation was identified by mechanical probing of the heart with a small glass pipette. According to our hypothesis, it was expected that CNFA would increase with rises as well as with decrease of LVEDP.

**PROTOCOL 4. CNFA: VOLUME EXPANSION.** To investigate the response of cardiac vagal C fibers to a more physiological and prolonged stimulus, volume expansion was performed in six rats as described in protocol 1. These rats were instrumented with a balloon catheter in the descending aorta, helping to ensure that the respective cardiac C fiber was mechanosensitive. Before volume expansion was started, the aortic balloon was pulled back to the femoral artery to avoid obstruction of the aortic outflow, which might have had confounding effects. LVEDP and CNFA were recorded as well as MAP, HR, and ECG. At the end of the experiments, rats were euthanized and the receptive field of the C fiber was sought by gently probing the cardiac surface with a glass pipette. CNFA was expected to increase with volume loading.

**PROTOCOL 5. CNFA: HYPTENSIVE HEMORRHAGE.** To investigate C-fiber responses to more physiological and prolonged decreases of LVEDP, rats (n = 6) underwent the hypotensive hemorrhage protocol of protocol 2 while LVEDP, CNFA, MAP, HR, and ECG were recorded simultaneously over 15 min. Rats were instrumented and fibers were screened as in protocol 4. The aortic balloon was pulled back before hemorrhage started.

After a recovery time of 3 min, the withdrawn heparinized blood was reinfused (at minute 18) to restore baseline blood pressure within 5–6 min. The reinfusion rate was adjusted by the observed increase of blood pressure. Receptive fields were sought after the rats were euthanized. CNFA was expected to increase with hemorrhage as well as with reinfusion, according to our hypothesis.

Single-fiber recordings in modified Langendorff heart preparation: protocol 6. The maneuvers used to stimulate mechanosensitive fibers by changing cardiac filling pressures in protocols 1–5 might all be capable of inducing changes in coronary perfusion. Therefore, we tried to investigate the effect of changes in CPP on cardiac C-fiber activity with the modified Langendorff heart preparation (n = 5).

Perfusion was started at constant pressure (70 mmHg) and temperature (37°C), and the preparation was allowed to stabilize until a constant HR was achieved. Because of the limitations of a continuously deteriorating preparation (edema formation, ischemia of surrounding tissues), only one mechanosensitive cardiac C-fiber unit was recorded during a short protocol. HR, CNFA, and CPP were recorded simultaneously. Forty seconds after recording was started, the perfusion pressure was abruptly increased to 100 mmHg and kept constant for 40 s before it was decreased to baseline level again. After another 40 s, CPP was decreased to 40 mmHg for a period of 40 s. Thereafter, CPP baseline was restored for another 40 s. As with changes in LVEDP (protocols 1–5), CNFA was expected to increase with both rise and fall of perfusion pressure.

**Data Analysis**

RSNA was recorded as millivolts times second. Measured activity (in μV·s) of each single animal was recalculated in consideration of individual amplifier settings and after subtraction of background noise level, i.e., the minimum activity recorded after ganglionic blockade (trimetaphan-camsylate, Arfonad; 10 mg/kg iv) and/or postmortem activity (average of 30 min). Baseline values of RSNA (μV·s; in protocols 1 and 2), CNFA (Hz; in protocols 4 and 5), MAP and LVEDP (mmHg), and HR (beats per minute, bpm) were averaged from 3-min periods before intervention (i.e., hemorrhage, volume expansion). Integration of RSNA bursts over time is a widely used method to subsume burst signal information (i.e., rhythmicity, burst amplitude, burst duration); however, comparability of absolute values between animals or groups is limited (11, 27). Therefore, percent changes from baselines (ARSA%) were analyzed. Baselines of CNFA (Hz; in protocols 3 and 6) were obtained from averaged 40-s periods sampled before application of respective stimuli. All sets of data to be analyzed were tested for normality with the Kolmogorov-Smirnov test (KST) to decide for parametric or nonparametric tests.

Differences of baseline parameters among groups and/or protocols could be ruled out with one-way ANOVA and Kruskal-Wallis test with post hoc all-pairwise comparisons according to the KST results and sample size. Intraindividual changes of MAP, LVEDP, HR, RSNA, and CNFA (consecutive 1-min intervals vs. baseline) induced by volume loading or hemorrhage (protocols 1, 2, 4, and 5) were analyzed with one-way repeated-measures ANOVA and Friedman’s test according to the KST results with post hoc multiple comparisons vs. baseline. Differences among groups (i.e., Vx vs. control, protocols 1 and 2) at corresponding 1-min intervals were analyzed with unpaired Student’s t-test and Mann-Whitney rank-sum test according to the KST results. CNFA and LVEDP data (protocols 4 vs. 5) were analyzed likewise.

CNFA data from protocol 6 at consecutive CPP (baseline vs. “high” vs. recovery 1 vs. “low” vs. recovery 2) were analyzed with Friedman’s test, whereas consecutive 10-s intervals were included in the analysis. CNFA data from protocol 3 were correlated to changes in LVEDP. Statistical analysis was performed at consecutive 1-mmHg increments and decrements by one-way repeated-measures ANOVA. Analysis was done separately for ramps to +4, +6, and +8 mmHg and −4, −6, and −8 mmHg, respectively. Furthermore, averaged CNFA at maxima of separate ramps was compared by ANOVA. Statistical significance was defined as P < 0.05. Data are given as means ± SE. SigmaStat 2.03 (Jandel) was used for data analysis.

**RESULTS**

Baseline values of MAP, HR (protocols 1–5), LVEDP (protocols 3–5), and RSNA (protocols 1 and 2) did not differ significantly between groups or protocols. Baseline MAP for all groups was 102 ± 8 mmHg, HR was 340 ± 29 bpm, and LVEDP was 4.6 ± 0.6 mmHg. Actually measured baseline RSNA was 83.9 ± 7.4 μV·s for all recordings. For further evaluation, percent changes from baseline activity were calculated.
Protocol 1. RSNA Recording: Volume Expansion ± Vx

In control rats (n = 6) MAP and HR were unaffected by volume loading (Fig. 1). RSNA continuously decreased down to −45 ± 8% from baseline within 4 min (P < 0.05). In rats that underwent bilateral cervical Vx before volume loading (n = 6), baseline MAP, HR, and RSNA were similar. Sympathoinhibition was abolished, whereas MAP and HR were unaffected by Vx. Furthermore, responses to baroreceptor loading and unloading were similar in Vx rats and controls, indicating integrity of baroreceptor afferents after Vx (data not shown). These findings indicate and confirm that vagal afferent fibers play a crucial role in this reflex response (3, 44).

Protocol 2. RSNA Recording: Hemorrhage ± Vx

In controls (n = 6) and bilaterally vagotomized rats (n = 6), baseline parameters did not differ from those in protocol 1 (Fig. 2); hemorrhage significantly decreased MAP by −50 ± 4 and −50 ± 6 mmHg, respectively. In controls, HR increased up to +49 ± 15 bpm from baseline (approximately +14.4%; P < 0.05) within 3 min but then decreased progressively toward resting values (+12 ± 12 bpm (approximately +3.5%) at minute 15). Within the first minute, RSNA increased up to +19 ± 8% (P < 0.05). Beginning at minute 2, RSNA decreased progressively to a minimum level of −39 ± 9% at minute 7, where it stabilized (P < 0.05 since minute 5). In vagotomized rats, HR increased continuously to +65 ± 18 bpm from baseline (approximately +19.1%; P < 0.05) within 3 min and stabilized at this level. RSNA again increased at minute 1 (+22 ± 7%; P < 0.05) as in controls. Then, however, it failed to decrease below baseline. Thus Vx abolished the second phase sympathoinhibition, indicating and confirming that vagal afferent nerve traffic plays a crucial role in the hemorrhage-induced reflex response as well.

Protocol 3. Cardiac C-Fiber Recording: Balloon Maneuvers

In 40 rats, 130 fibers were screened. After exclusion of noncardiac fibers, fibers from 32 rats were tested for mechanosensitivity according to our inclusion criteria. The mean increase in activity due to aortic obstruction (LVEDP +6 mmHg) was 5.7 ± 1.1 Hz (Fig. 3). Fibers that did not react to aortic obstruction—classified as being not mechanosensitive—were excluded as well. Finally, 70 fibers from 25 rats were included in the analysis. Changes in LVEDP were induced by graded inflation of the caval vein and the aortic balloon (ramps to ±4, ±6, and ±8 mmHg, respectively). Between 8 and 12 separate ramps per fiber could be done. The mean conduction velocity of these fibers was 0.84 m/s (range 0.6–1.6 m/s), thus meeting criteria of C-fiber conduction (37, 41). The mean resting activity of these fibers expressed in spikes per second was 2.64 ± 0.52 Hz. Increase of LVEDP by +4, +6, and +8 mmHg from baseline increased CNFA by 4.30 ± 0.55 (approximately +163%), 5.60 ± 0.61 (approximately +212%), and 14.60 ± 3.08 (approximately +553%) Hz, respectively. These increases in CNFA were significantly different compared with respective baselines as well as between stimuli (+4 vs. +6 vs. +8 mmHg; P < 0.05). Decrease of LVEDP by 4, 6, and 8 mmHg below baseline again increased CNFA by 3.98 ± 0.48 (approximately +151%), 5.95 ± 0.75 (approximately +225%), and 15.90 ± 2.85 (approximately +602%) Hz, respectively (P < 0.05; baseline vs. maximum response for each stimulus as well as −4 vs. −6 vs. −8 mmHg). These data indicate that these mechanosensitive vagal C-fiber afferents increase their firing rate in response to LVEDP changes in either direction. The rate of change of LVEDP (mmHg/s) differed considerably between respective ramps (as well as compared with the physiological experiments; see Fig. 4). It was approximately twice as high in the ±8-mmHg ramps than in the ±4-mmHg ramps because maximum changes were always obtained within the same time...
Fig. 3. Responses of single afferent C fibers (n = 70) due to rapid changes of left ventricular end-diastolic pressure (LVEDP). ∆CNFA is plotted against ∆LVEDP. Gray boxes ("hold") in A–C symbolize the period (12–15 s) when balloons were held inflated to obtain "peak" values. Horizontal arrows symbolize the period (7–12 s) when balloons were inflated to change LVEDP. A: pooled data from "ramps" to +4 and −4 mmHg (■). ∆CNFA increased virtually linearly due to LVEDP changes. B: ramps to +6 and −6 mmHg (●). As in A, ∆CNFA increased due to LVEDP changes. Peak ∆CNFA was significantly higher than in A but lower than in C. C: ramps to +8 and −8 mmHg (▲). As in A and B, a virtually linear increase of ∆CNFA is seen. Peak ∆CNFA response was significantly higher than in A and B. *P < 0.05, ∆CNFA vs. baseline at 1-mmHg increments and decrements. †P < 0.05, ∆CNFA peak values, ±4 mmHg (A) vs. ±6 mmHg (B) vs. ±8 mmHg (C). D: sensitivity of CNFA response to the rate of change in LVEDP. ∆CNFA was plotted against ∆LVEDP (lower y-axis, large symbols) as in A and C. Large gray symbols represent data from ±4-mmHg ramps; the corresponding solid line (A) denotes the linear regression curve [r² = 0.93; slope a (Hz/mmHg) = 0.78 for positive, −0.85 for negative LVEDP]. Large solid symbols represent data from ±8-mmHg ramps; the corresponding solid line (B) denotes the linear regression curve [r² = 0.92; a = 1.58 or −1.40]. Furthermore, ∆CNFA was plotted against time spans, i.e., 7, 9.5, and 12 s (upper y-axis, small symbols). Small gray symbols represent ±4-mmHg ramps; the corresponding dotted line (C) denotes the regression curve [r² = 0.77; a (Hz/s) = 0.31 and −0.34]. Small solid symbols represent ±8-mmHg ramps with the corresponding black dotted linear regression curve (D; r² = 0.78; a = 1.27 and −1.21, respectively). These data indicate "rate sensitivity" of the C-fiber response.

We did linear regression analysis for respective ramps (+4, +8, −4, and −8 mmHg; inflation/deflation periods only) using a simple equation [\( Y = a \cdot X \), where \( Y \) is ∆CNFA, \( X \) is ∆LVEDP, and \( a \) is the slope (Hz/mmHg)]. The coefficient of correlation \( r^2 \) was 0.93 ± 0.01, indicating clear-cut linearity of response. The slope in the ±8-mmHg ramps was nearly twice that of the ±4-mmHg ramps (\( P < 0.01 \); see Fig. 3D). Furthermore “time-based” analysis was done: CNFA changes were plotted against mean ramp duration (i.e., 9.5 s) and extremes (i.e., 7 and 12 s), and linear regression revealed significant differences of slopes (Hz/s; \( P < 0.005 \); see Fig. 3D). Thus the afferent responses due to rapid stimulation appeared to be rate sensitive. Furthermore, a substantial increase in CNFA was observed already with minor changes in LVEDP. However, slope estimation was based on an “ex post” analysis of experiments that were not specially designed for investigation of rate sensitivity. Therefore, this finding must be interpreted with caution.

Protocol 4. Cardiac C-fiber Recording: Volume Expansion

In six rats each, a single cardiac mechanosensitive C fiber was identified. Resting CNFA was 1.00 ± 0.82 Hz. Rats were given an intravenous volume expansion as in protocol 1. LVEDP, CNFA, MAP, HR, and ECG were recorded simultaneously. LVEDP increased progressively to +10.00 ± 0.22 mmHg from baseline (\( P < 0.05 \); Fig. 5) until minute 7 and then fell continuously to +4.50 ± 0.50 mmHg at minute 15, still
much higher in plotted against time. It is clearly shown that the rate of change of LVEDP was
and well above baseline level (P < 0.05). As in protocol 1, MAP, HR, and ECG were not affected by this procedure (data not shown). CNFA increased to a maximum of +10.33 ± 3.68 Hz (approximately +1.033%) at minute 3 (P < 0.01) and then decreased continuously to +4.00 ± 0.73 Hz (approximately +450%; P < 0.05) at minute 9, where it stabilized. As expected, the increase in LVEDP was accompanied by an increase in CNFA. However, maximum CNFA was already reached at ~55% of maximum ΔLVEDP. Vice versa, at maximum ΔLVEDP, CNFA had already returned to ~55% of maximum CNFA. Thus there was no clear-cut linearity between CNFA and LVEDP with prolonged stimulus.

Protocol 5. Cardiac C-Fiber Recording, Hemorrhage, and Reinfusion

In six animals, a single cardiac mechanosensitive C-fiber was again identified and CNFA was recorded simultaneously with LVEDP, MAP, and HR. Resting CNFA was 0.83 ± 0.40 Hz. Other baseline parameters also did not differ from those in protocol 4. Hemorrhage significantly decreased LVEDP to −5.38 ± 1.22 mmHg from baseline at minute 7 (P < 0.05; Fig. 6). It then slowly returned toward baseline but was still significantly below at minute 15 (~2.50 ± 0.50 mmHg; P < 0.05). CNFA again significantly increased by up to 10.17 ± 1.85 Hz (approximately +1,225%) at minute 3 (P < 0.003). Activity then slowly decreased to +2.50 ± 0.34 Hz (approximately +301%), still well above baseline level (P < 0.01).

At minute 18 blood was reinfused. LVEDP increased from 4.00 ± 0.80 mmHg below baseline to a maximum of +1.30 ± 0.70 at minute 27. At minute 18 CNFA was still above baseline (+2.33 ± 0.33 Hz; P < 0.05), increased to a maximum of +6.67 ± 1.15 Hz (approximately +233%) at minute 23 (P < 0.005), and returned to baseline until minute 37.

Thus hemorrhage decreased LVEDP, and this was accompanied by an increase in CNFA according to our hypothesis. With reinfusion, LVEDP normalized and CNFA again increased comparable to volume expansion. Again, there was no clear-cut linearity between LVEDP and CNFA. CNFA maxima were reached before maxima in ΔLVEDP. At ΔLVEDP maxima, CNFA had already returned to ~57–60% of the maximum response.

Protocol 6. C-Fiber Recordings in a Modified Langendorff Heart Preparation

We were able to investigate five cardiac mechanosensitive C-fibers in five separate preparations. HR and CNFA were recorded simultaneously over 200 s as well as perfusion pressure, which was changed in steps of 30 mmHg every 40 s (baseline, high, recovery 1, low, recovery 2).
The LV. No receptive fields could be detected in the atria. Experiments showed that the receptive fields were clustered in the LV, but no atrial receptive field was found. Thus mechanosensitive cardiac C-fiber activity was uniformly increased by short-term as well as prolonged mechanical stimuli able to increase or decrease LVEDP (i.e., volume expansion/aortic obstruction and hemorrhage/caval vein obstruction, respectively). This confirms our recent finding (47) that increasing LVEDP by aortic obstruction increases single vagal C-fiber activity. Furthermore, on first sight, our findings seem to be in good accordance with those from a feline study by Oberg and Thoren (31) showing that severe hemorrhage or caval vein occlusion to mimic rapid blood withdrawal paradoxically increased single vagal C-fiber activity.

However, in the Langendorff heart, we found that CNFA reacted directly proportionally to changes in CPP; an increase in CPP led to an increase in CNFA, and vice versa. To our knowledge, those responses have not been described previously. The receptive fields of recorded cardiac C fibers were clustered in the LV, but no atrial receptive field was found.

**DISCUSSION**

Volume expansion-induced sympathoinhibition is mediated by cardiac C-fiber activation (3, 24), seemingly because of increasing cardiac filling pressure, because C-fiber activity has been shown to be closely related to LVEDP (47). This is in good accordance with the opinion that cardiac afferent fibers act mainly as tension receptors within the cardiac wall (38). Our study was done primarily to elucidate the role of cardiac C-fiber afferents in the context of hemorrhage-induced sympathoinhibition where LVEDP is decreased. We intended to systematically investigate the effects of central volume load and depletion—due to physiological and very rapid measures—on cardiac vagal afferents influencing the reflex control of RSNA. We hypothesized that cardiac afferent C-fiber activity (CNFA) is augmented along with increases as well as decreases in LVEDP. Because the maneuvers used to modulate LVEDP supposedly had impact on coronary perfusion, the modified Langendorff heart preparation was used to investigate CPP as a putative stimulus for cardiac C-fiber activation.

We found that bilateral cervical Vx abolished reflex inhibition of RSNA due to volume expansion and hemorrhage, thus confirming the voluminous previous work in numerous species that vagal afferents influence RSNA in both of these reflex responses (3, 16, 23, 36, 44). Furthermore, we found that CNFA was uniformly increased by short-term as well as prolonged mechanical stimuli able to increase or decrease LVEDP (i.e., volume expansion/aortic obstruction and hemorrhage/caval vein obstruction, respectively). This confirms our own recent finding (47) that increasing LVEDP by aortic obstruction increases single vagal C-fiber activity. Furthermore, on first sight, our findings seem to be in good accordance with those from a feline study by Oberg and Thoren (31) showing that severe hemorrhage or caval vein occlusion to mimic rapid blood withdrawal paradoxically increased single vagal C-fiber activity.

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Because of the method used for identification of mechanosensitive C fibers there might be, however, some bias in favor of ventricular fibers. Therefore, our interpretations of the results refer exclusively to ventricular vagal C fibers.

C-fiber recordings were done in anesthetized and paralyzed animals. To obtain similar experimental conditions throughout the study, volume expansion and hypotensive hemorrhage were tested under these conditions. With hemorrhage, we found a biphasic pattern of the reflex response of HR and RSNA nearly similar to that previously described in the conscious state in different species (18, 35). Therefore, we assumed that the protocols as well as the anesthetic regimen would be adequate to investigate cardiac C-fiber responsiveness due to hemorrhage and volume expansion. Balloon maneuvers used to simulate central volume loading and depletion were adapted from a previous study (47), served as a “positive control” for volume expansion and hemorrhage, respectively, and allowed us to investigate the effects of any LVEDP change within the same fiber repeatedly.

Basically, the task of reflex mechanisms is maintenance of stable physiological conditions, which is mostly accomplished by negative-feedback control loops. Mechanosensitive cardiac C fibers play a crucial role in volume homeostasis via RSNA. Increase in RSNA favors volume retention; decrease has the opposite effect. Volume overload and depletion find their expression in high and low LVEDP values, respectively. Thus sympathoinhibition (RSNA reduction) in the setting of severe volume depletion seems to be a paradox. In terms of cybernetics, RSNA could be defined as the “manipulated variable.” The “controller” would be CNFA, and the “command variable” could be LVEDP. However, because the CNFA response turned out to be only directly proportional to the absolute value of LVEDP regardless of its algebraic sign, LVEDP might be a weak surrogate rather than an applicable command variable. Otherwise, CNFA should decrease rather than increase in the setting of hypotensive hemorrhage. The classic hypothesis for this paradoxical activation of cardiac ventricular C fibers comes from the feline study by Oberg and Thoren (31). Vigorous contraction of the myocardium around an “empty ventricle,” thereby squeezing vagal afferent ventricular fiber endings, was suggested, i.e., increased inotropic state combined with low ventricular filling might constitute the intrinsic stimulus for CNFA. In that study the firing rate of ventricular fibers increased all of a sudden when blood volume became critically low. In rats we also found increase in CNFA with hemorrhage and caval vein obstruction; however, we found that CNFA increased substantially during the initial falling phase of LVEDP. This was observed with caval vein obstruction as well as with hemorrhage. With aortic obstruction and volume expansion CNFA response was virtually identical. Then, with lasting stimulation, CNFA stabilized at a higher baseline (“resetting”). The underlying “adaptive” mechanisms might be comparable to those suggested for arterial baroreceptor modulation, i.e., viscoelastic relaxation, Na⁺ pump and K⁺ channel activation, and paracrine and autocrine factors (6). With rapid stimulation there was also some indication of time-dependent C-fiber behavior. The slope of the CNFA curve (Hz/mmHg or Hz/s) to ±8-mmHg ramps was clearly higher than the slope of the CNFA curves to ±4-mmHg ramps (see Fig. 3D). Thus CNFA seemed to be sensitive to the rate of change in LVEDP in these experiments. However, we think that this finding must be interpreted with caution because there was substantial variation in the time interval (i.e., 7–12 s) to obtain respective maximum changes in LVEDP. Furthermore, it is based on an ex post analysis of data obtained from an experimental approach that was not specifically designed for the investigation of “rate sensitivity.” This time- and/or rate-dependent behavior (at least with rapid LVEDP changes), as well as the fact that CNFA substantially increased already with minor LVEDP changes (rapid as well as prolonged), seems to be—at least in part—contradictory to the classic hypothesis that vigorous contraction of the empty heart constitutes the intrinsic stimulus for ventricular C-fiber activation. Further doubt on this hypothesis came from a study in dogs that showed that low ventricular filling with increased inotropic state was unable to induce reflex vasodilatation (15). Therefore, it seems questionable that LVEDP (as a measure of cardiac filling) alone constitutes an intrinsic stimulus for CNFA in hemorrhage.

Interestingly, data from the Langendorff heart preparation revealed direct proportionality between CPP and CNFA. Therefore, CPP might be an applicable command variable rather than LVEDP. CNFA responses due to CPP changes were smaller than those induced by changes in cardiac filling. Nevertheless, this finding might well be physiologically relevant because the difference could just be related to stimulus intensity. However, this CPP effect also does not explain well the CNFA response due to hemorrhage: CPP can be estimated by the gradient between diastolic blood pressure and LVEDP. In hemorrhage, however, the decrease in diastolic pressure is much more pronounced than the decrease in LVEDP. Thus CPP decreases, and one would expect CNFA to decrease if CPP was the intrinsic stimulus, according to our findings from the Langendorff heart experiments. In fact, the opposite response occurred with hemorrhage as well as with caval vein obstruction. Therefore, neither LVEDP nor CPP alone fully accounts for ventricular C-fiber activation in hemorrhage. With volume loading and aortic obstruction, however, LVEDP as well as CPP fulfill the properties of an applicable command variable.

Although CPP might not constitute the intrinsic stimulus for CNFA in the setting of hemorrhage there is some good evidence, in accordance with our finding that CPP, nevertheless might be an important stimulus for ventricular C-fiber activation. In dogs, increased CPP constitutes a stimulus to induce reflex vasodilatation that is mediated by ventricular and/or coronary mechanoreceptors (1). Electrophysiological techniques and a very sophisticated perfusion circuit that allowed independent change of ventricular, coronary, and carotid pressures revealed that coronary pressure seems to be the predominant stimulus for activation of cardiac mechanosensitive af-
ferents. Changes in ventricular pressures (LVEDP >> peak systolic pressure) had a comparably small effect that was only seen with nonphysiologically high pressures. Therefore, it was argued that ventricular receptors would only play a role with gross distension of the cardiac wall, where they might have protective effects. However, in rats, we found an increase in CNFA also with minor physiological changes in LVEDP. Thus in this species ventricular cardiac C fibers might have protective as well as regulative functions under normal conditions. In dogs, increases in CPP—in a range that induced reflex vasodilatation—increased discharge of myelinated fibers (“A”) fibers, whereas nonmyelinated C fibers were not sensitive to such moderate CPP changes (14). In rats, we found that C-fiber activity changed along with rather moderate CPP changes. A-fibers were not investigated in our study.

Furthermore, it is widely accepted that increasing CPP as well as flow augments cardiac contractility in isolated, perfused hearts, a finding termed the “Gregg phenomenon” that seems to be mediated by stretch-sensitive ion channels (20, 25). Thus CNFA in the Langendorff heart might have been influenced by CPP and/or coronary flow during diastole as well as by contractility. This consideration might constitute a feasible explanation for the widely accepted finding that firing of cardiac mechanosensitive C fibers takes place mainly during systole while obviously being set by diastolic events (38–40). However, increases in contractility (i.e., inotropic state) by dobutamine or by electrical stimulation in dogs failed to induce significant reflex responses (15); thus contractility itself might not constitute a major stimulus for cardiac afferent discharge. However, because sympathetic nerve activity is regulated differentially in terms of target organs and tissue (29), lack of reflex vasodilatation does not necessarily mean that cardiac afferent discharge is not influenced by changes in contractility.

With decreasing LVEDP, passive compression of blood vessels within the cardiac wall might be decreased. Therefore, it might be tempting to speculate that coronary flow might be favorably altered, thus increasing CNFA. However, CPP and coronary flow are not necessarily interchangeable. Moreover, it has been clearly shown in a study in dogs and primates that coronary flow is reduced in the setting of hypotensive hemorrhage whereas coronary vascular resistance was increased, which is an active function of vascular smooth muscle cells (42).

Nevertheless, when coronary pressure is changed alterations in coronary flow are inevitable. Therefore, we believe that the effect of changing coronary pressure on CNFA reflects a mixture of direct mechanical, pressure-derived effects (e.g., stretch) as well as effects secondary to flow-mediated events (e.g., altered oxygen or substrate delivery), particularly because most ventricular C fibers in rats have been shown to be at least bimodal (47), meaning that one and the same fiber might be susceptible to mechanical as well as chemical stimuli. Furthermore, the effect of one kind of stimulus might be influenced by another. Thus humoral factors might well constitute the “missing link” between cardiac C-fiber activation and hemorrhage. This opens the discussion for a variety of humoral factors that might influence cardiac afferent fiber activity. In fact, it has been shown that subthreshold stimulation of 5-HT3 receptors blunted the RSN response due to volume expansion (44) and that this might be due to 5-HT3 receptor-mediated desensitization of mechanosensitive afferent C fibers (26). The opioid receptor antagonist naloxone given intravenously has been shown to abolish hemorrhage-induced renal sympathoinhibition (5, 21). Furthermore, vasopressin has been shown to strengthen or blunt hemorrhage-induced sympathoinhibition via V1 and V2 receptors, respectively. Whether endogenous opioids and vasopressin or other humoral factors directly influence the sensory afferent nerve, as shown for the 5-HT3 receptor agonist phenylbiguanide, is not clear yet.

Humoral factors might also be involved in the adaptive CNFA response that we saw with prolonged stimuli. Such factors might be secreted rapidly because of counterregulatory mechanisms, thus desensitizing mechanosensitive C fibers.

In conclusion, we were able to show that LV vagal C fibers increase activity in response to any change in LVEDP but react directly proportionally to CPP. Furthermore, we found a V-shaped response of CNFA due to LVEDP changes as well as time-dependent adaptation. At least in the context of volume expansion, both these mechanical factors might constitute an intrinsic stimulus for C-fiber activation.

However, neither LVEDP nor CPP alone fully accounts for C-fiber activation with central volume depletion. Therefore, the intrinsic stimulus for C-fiber activation in hemorrhage might rather consist of different “mechanical” factors (e.g., LVEDP, CPP, coronary flow, smooth muscle contraction of coronary vessels, cardiac contractility) as well as humoral factors that interplay to activate possibly branched polymodal fibers, originating from distinct receptive fields. Further work is needed to elucidate the intrinsic sensory properties and receptive fields of cardiac vagal afferents because such afferent systems might also constitute interesting pharmacotherapeutic targets.

REFERENCES

CARDIAC C FIBERS IN HEMORRHAGE AND VOLUME EXPANSION


